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(71) Applicant: Cedars-Sinai Medical Center
8700 Beverly Boulevard
Los Angeles California 90048(US)

(72) Inventor: Rubinstein, Alan
803 North Bedford Drive
Beverly Hills California 90210(US)

(74) Representative: Hansen, Bernd, Dr.rer.nat. et al,
Hoffmann, Eitle & Partner Patentanwälte
Arabellastrasse 4
D-8000 München 81(DE)

(54) **A method for the heat treatment of plasma of plasma fractions and compositions obtained thereby.**

(57) Heat treatment of dry, for instance, lyophilized plasma compositions particularly fractions containing Factor VIII concentrates inactivates undesirable micro-organisms such as any hepatitis virus. Subsequent reconstitution is effected by adding sterile water until solubilization. Therapeutically and diagnostically useful products and procedures are achieved, for instance permitting transfusion of plasma to hemophiliacs without fear of conveying hepatitis.

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A Method for the Heat Treatment of Plasma or Plasma Fractions and Compositions obtained thereby

The present invention relates generally to heat treated plasma. In particular the invention is directed to heat treated fractions which may be heated in lyophilized form for the purpose of inactivating undesirable microorganisms and viruses, such as hepatitis virus present in the fractions.

Utilization of clotting factor concentrates obtained from fractionated blood plasma for the purpose of intervening therapeutically with respect to inherited bleeding disorders such as hemophilia is severely compromised as a consequence of the inordinate risk posed to the hemophiliac patient by the presence of hepatitis virus in the concentrates. For example, commercial Factor VIII and IX concentrates are typically employed to increase the clotting ability of a hemophilia victim's blood, but these concentrates are prepared from pools of plasma contributed by thousands of donors and contain the inherent hepatitis risk of a like number of single unit transfusions. As McCullen and Zuckerman have shown, see Journal of Medical Virology, Vol. 8, No. 29 (1981), despite stringent screening of individual donors for hepatitis B surface

antigens (HBsAg), such plasma pools clearly transmit both hepatitis B and non-A, non-B hepatitis.

Hepatitis transmission by albumin and other heat-stable plasma components unrelated to blood coagulation has heretofore been prevented by heating the plasma components in solution at temperatures of 60° C. for ten hours. Similar attempts to heat clotting factor concentrates in solution, by way of contrast, have been shown to markedly reduce or eliminate clotting factor activity in the concentrates and thus do not appear to offer a viable solution to the problem of hepatitis transmission associated with conventional hemophiliac therapy. More recently, highly purified Factor VIII precipitate has been dissolved in a solution of sucrose glycine and heated for ten hours at 60° C. Although the Factor VIII concentrate subsequently derived from the heated precipitate does retain clotting factor activity, the yields obtained using this approach are very low, e.g., about 8%. See Heimburger, et al., Hemostatis, Vol. 10 (supplement 1), p. 204 (1981) and Heimburger, et al., Blut, Vol. 44, p. 249-251 (1982).

As a net result, the prior art to date does not furnish any means for effectively inactivating hepatitis virus present in clotting factor concentrates nor does the prior art teach a means for effectively inactivating hepatitis virus present in clotting factor concentrates nor does the prior art teach a means for preventing the transmission of hepatitis virus to patients undergoing therapy with clotting factor concentrates.

According to the present invention the heat treating of substantially dry compositions VIII inactivates, reduces or eliminates the infectivity of the microorganisms, such as viruses present therein without reducing clotting factor activity.

Further, the heat treating of substantially dry clotting factor concentrates including Factor VIII inactivates any hepatitis virus present therein permitting a substantial yield of concentrate without significant reduction of clotting factor activity in the concentrate.

Also by the present invention is included the heat treating of clotting factor concentrates containing Factor VIII wherein the concentrates are first prepared in lyophilized form to enhance the stability of the concentrates during the heating process.

Heat-treated lyophilized plasma fractions preferably produce a composition and a vaccine effective against both hepatitis B virus and non-A, non-B hepatitis virus.

These and other aspects of the present invention are achieved by lyophilizing either whole plasma or plasma fractions including Factor VIII concentrate, and also factors such as Factor IX concentrate, fibrinogen and cryoprecipitate and thereafter subjecting the lyopholized whole plasma or plasma fractions to elevated temperatures for varying periods of time.

The ability to isolate clotting factors present in human blood has been indispensable in understanding the pathology of hemophilia and other inherited bleeding disorders. Concommitantly, the discovery of plasma fractionation schemes for obtaining practical quantities of clotting factor concentrates has enabled medical science to utilize the clotting factor concentrates as therapeutic tools in treating bleeding disorders. Transfusion therapy employing Factor VIII and Factor IX concentrates in particular has proven quite successful in ministering to hemophiliac patients. Unfortunately, the risk of hepatitis transmission due to the large number of plasma donors required for commercial production of clotting factor concentrates remains as the one serious drawback associated with transfusion therapy. A typical

plasma fractionation scheme, disclosed in Seminars in Thrombosis and Hemostatis, Vol. VI, No. 1, p. 4 (1979), yields cryoprecipitate and supernate, the former fraction constituting a source of both Factor VIII concentrate and fibrinogen and the latter fraction constituting a source of Factor IX concentrate in addition to Factors II, VIII, and I concentrates. As Gerety and Eyster have demonstrated in "Hepatitis Among Hemophiliacs", Non-A, Non-B Hepatitis, p.103-106 (1981), hepatitis B virus initially present in whole plasma is distributed to the Factor VIII and Factor IX derivatives during the plasma fractionation process. As also demonstrated by Maynard and Bradley, "Transmission by Blood Products", Non-A Non-B Hepatitis, p. 78-79 (1981), non-A, non-B hepatitis exists in both Factor VIII and Factor IX derivatives. Previous attempts to heat-treat clotting factor concentrates in solution for the purpose of inactivating hepatitis virus have been ineffective. The development of techniques for lyophilizing clotting factor derivatives, however, has opened a new avenue of exploration with regard to stabilizing clotting factor derivatives during the heat treating process, in turn establishing a means for inactivating hepatitis virus present in the clotting factor derivatives without destroying clotting factor activity.

Test Procedures for Verifying
Retention of Clotting Factor Activity

Paired samples of various lyophilized plasma fractions, each such pair having identifcal lot numbers, were received from several manufacturers. The samples generally weighed less than 100 g and were packaged in vials having volumes of 60 ml to 90 ml. One sample in each pair was heated, either by placing the sample vial in a water bath or dry oven at a predetermined temperature under room pressure for a predetermined period of time, or by placing the lyophilized material itself in a dry oven

without the vial present. The remaining sample in each pair served as a control and was refrigerated at 4°C - 6°C during the heat-treating process. Following heat treatment, both the control and heat-treated lyophilized samples were reconstituted with sterile water. Reconstitution was generally carried out according to manufacturer's specifications, although the solubility of some haat-treated samples was markedly improved by increasing the amount of sterile water used during reconstitution over that recommended by the manufacturer. In vitro Factor VIII and Factor IX assays were performed using a one-stage manual fibrometer method at dilutions ranging between 1:40 and 1:400 to obtain a measure of Factor VIII and Factor IX clotting activity. In vitro recovery of fibrinogen following reconstitution of both the control and heat-treated lyophilized fibrinogen samples was measured in a similar fashion. In some of the experiments, reconstituted plasma fractions were observed for light transmission at 580 nm in a Beckman Model 25 Spectrophotometer. Agarose gel electrophoresis with an ICL immunoelectrophoresis plate was carried out for several of the Factor VIII and Factor IX paired samples, using goat anti-human serum supplied by Hyland Diagnostics as a standard. The plates were specifically electrophoresed by a Buchler power supply set at 25 ma for 35 minutes. Upon completion of the electrophoresis, the plates were incubated in antisera for 18 to 24 hours and examined under indirect light. Panagell electrophoresis with a Worthington Diagnostics plate was carried out on additional paired samples of Factor VIII concentrate, using a Biorad water-cooled electrophoresis cell.

Further in vitro experiments were performed by heating lyophilized samples of Factor VIII concentrate in a water bath at room temperature and at predetermined temperatures for predetermined periods of time. Factor $VIII_{Ag}$ was then determined using the method described by Laurell, "Electroimmuno Assay," The Scandinavian

Journal of Clinical and Laboratory Investigation, Vo. 29, pp. 21-37 (1972). Factor VIII results were calculated for dilutions of 1:40, 1:80, 1:100 and 1:200 by plotting the height of the rockets of the Laurell standard curve against the percentage of dilution. Unknowns were expressed as a percentage of normal, based on the rocket heights of the unknowns in the standard curve.

In vivo recovery of clotting factor activity for both heat-treated Factor VIII and Factor IX concentrates was measured by injecting reconstituted, heat-treated lyophilized Factor VIII and Factor IX concentrates respectively into hemophilia A and hemophilia B dogs. A heat-treated, lyophilized Factor IX concentrate was also injected into a control dog. Laboratory parameters including Hct, serum protein, WBC, platelet count, blood smear, respiration rate, body temperature, pulse and clotting factor activity were subsequently ascertained for each of the animals at various intervals following the injections.

Results of the in vitro testing performed on Factor VIII concentrate are summarized in Tables I and II:

TABLE I

Measurements of Clotting Factor Activity Following Heat-Treatment of Lyophilized Factor VIII Concentrate

Lot *	Temp.	Time	Dilution	% Activity
A C-1081	Control	--	1:20	
C-1081	Control	--	1:40	Approximately 10% decrease in activity was observed for heat-treated samples relative to the control.
C-1081	Control	--	1:80	
"	60°C.	10 hr.	1:20	
"	"	"	1:40	
"	"	"	1:80	
A NC-8247	Control	--	1:40	1438
"	"	--	1:80	1697
"	62°-64°C.	16.33 hr.	1:40	1215
"	"	"	1:80	1360
B AHF-355	Control	--	1:40	1912
"	"	--	1:80	1600
"	"	--	1:160	1312
"	64°C	20 hr.	1:40	1080
"	"	"	1:80	1072
"	74°C.	17 hr.	1:40	1144
"	"	"	1:80	1024
"	"	"	1:160	864
"	76°C.	17 hr.	1:40	1040
"	"	"	1:80	976
B 347	Control	--	1:100	1180
"	"	--	1:200	100
"	83°C.	24 hr.	1:100	100
"	"	"	1:200	100
"	85°C.	24 hr.	1:100	1

Table I Continued

Lot	Temp.	Time	Dilution	% Activity
B 347	95°C.	7 hr.	1:100	1
"	97°C.	7.5 hr.	1:200	1
C A1-0470	Control	--	1:100	912
"	75°C.	20 hr.	1:100	2076
C A1-1080	Control	--	1:200	2080
"	"	--	1:400	1920
"	80°C.	24 hr.	1:200	1380
"	"	"	1:400	1360
C A1-1120	Control	--	1:40	2800
"	"	--	1:80	2032
"	78°C.	21 hr.	1:40	1592
"	"	"	1:80	1392
"	80°C.	20 hr.	1:40	1176
"	"	"	1:80	1600
"	90°C.	12 hr.	--	Clotted Specimen
"	100°C.	1.5 hr.	1:40	1248
"	"	"	1:80	1264
C A1-1150	Control	--	1:40	2176
"	"	--	1:80	2480
"	"	--	1:100	1870
"	65°C.**	26.33 hr.	1:40	1592
"	"	"	1:80	1520
"	83°C.	24 hr.	1:100	730
"	"	"	1:200	620
"	85°C.	24 hr.	1:100	1000
"	"	"	1:200	1160
"	90°C.	10 hr.	1:40	1032
"	"	"	1:80	1056
"	95°C.	7 hr.	--	Clotted Specimen
"	97°C.	7.5 hr.	1:100	80
"	"	"	1:200	100
"	100°C.	10 hr.	1:40	88
"	"	"	1:80	48

Table I Continued

Lot	Temp.	Time	Dilution	% Activity
C A1-1160	Control	--	1:100	3680
"	"	--	1:200	3420
"	"	--	1:400	3200
"	78°C.	24 hr.	1:100	2420
"	"	"	1:200	1520
"	"	"	1:400	1440
"	78°C.	24 hr.	1:200	1720
"	"	"	1:400	1680
"	80°C	22 hr.	1:200	1400
"	"	"	1:400	1360
"	100°C.	7 hr.	1:200	1760
"	"	"	1:400	1760
C A1-2120	Control	--	1:100	8 6
"	110°C****	1.5 hr.	1:100	18
C A1-2531	Control	--	1:200	3500
"	"	--	1:400	2700
"	85°C.	20 hr.	1:200	46
"	"	"	1:400	43

Note: All times and temperatures are approximate. Following heat treatment at higher temperatures, amounts of sterile water in excess of manufacturer's recommendations were added to some concentrates until solubilization was visually confirmed.

* A lots were manufactured by Cutter Laboratories; B lots were manufactured by Michigan Red Cross; C lots were manufactured by Alpha Therapeutics.

** Heat-treated in a dry oven (sample removed from vial)

*** Heat-treated in a dry oven (sample contained in vial)

TABLE II

Determination of Factor VIII$_{Ag}$ Following Heat-Treatment of Lyophilized Factor VIII Concentrate

Lot	Temp	Time	Dilution	Rocket Height (mm)	%Ag
B AHF-355	Control	--	1:40	35	3720
"	"	--	1:80	22	4080
"	64°C.	20 hr.	1:40	39	4240
"	"	"	1:80	26	5120
"	74°C.	17 hr.	1:40	40	4400
"	"	"	1:80	26	5120
C Al-1120	Control	--	1:100	15	4700
"	90°C.	12 hr.	1:100	0	0
C Al-1150	Control	--	1:200	13	7200
"	83°C.	24 hr.	1:200	12	6200
"	85°C.	24 hr.	1:200	15	9400
"	97°C.	7.5 hr.	1:200	0	0

Note: All times and temperatures are approximate. Following heat treatment at higher temperatures, amounts of sterile water in excess of manufacturer's recommendations were added to some concentrates until solubilization was visually confirmed.

* B lots were manufactured by Michigan Red Cross; C lots were manufactured by Alpha Therapeutics.

Results from the testing of Factor IX concentrate are summarized in Table III:

TABLE III

Measurements of Clotting Factor Activity Following Heat-Treatment of Lyophilized Factor IX Concentrate

Lot	Temp.	Time	Dilution	% Activity
*				
A 9-C0044	Control	--	1:40	616
"	"	--	1:80	1200
"	"	--	1:200	2400
"	"	--	1:400	3520
"	100°C.	4 hr.	1:40	520
"	"	"	1:80	1104
"	100°C.	12 hr.	1:200	1680
"	"	"	1:400	2480
"	110°C.*	13 hr.	1:400	1640
"	" **	"	1:800	2560
"	122°C.	12 hr.	1:200	340
"	" **	"	1:400	480
"	132°C.	12 hr.	1:200	12
"	"**	"	1:400	24
A NC9055	Control	--	n/a	2600
"	100°C.	0.5 hr.	n/a	2350

Note: All times and temperatures are approximate.

Following heat treatment at higher temperatures, amounts of sterile water in excess of manufacturer's recommendations were added to some concentrates until solubilization was visually confirmed.

* A lots manufacutured by Cutter Laboratories.

** Heat treated in a dry oven.

Results of the testing performed on fibrinogen concentrate are summarized in Table IV:

TABLE IV

Recovery of Fibrinogen Following Heat-Treatment of Fibrinogen Concentrate in Lyophilized Form

Lot	Temp.	Time	Dilution	Recovery(mg/dl)
*D-003678	Control	--	1:20	400
"	"	--	1:40	680
"	60°C.	10-11 hr.	1:20	660
"	"	"	1:40	680
"	Control	--	1:10	195
"	"	--	1:20	760
"	"	--	1:40	700
"	60°C.	10 hr.	1:10	105
"	"	"	1:10	225
"	"	"	1:20	250
"	60°C.	17 hr.	1:10	190
"	"	"	1:20	220
*E	Control	--	1:40	1280
"	"	--	1:80	1280
"	60°C.	10 hr.	1:40	1520
"	"	"	1:80	1320
"	60°C.	10 hr.	1:40	1860
"	"	"	1:80	1520
"	65°C.	10 hr.	1:40	1640
"	"	"	1:80	1400
*E	65°C.	23 hr.	1:40	1420
"	"	"	1:80	1320
"	Control	--	1:40	1048
"	"	--	1:80	1064

Table IV Continued

Lot	Temp.	Time	Dilution	Recovery(mg/dl)
*E	100°C.	3 hr.	1:40	788
"	"	"	1:80	784
"	254°F**	3 hr.	1:5	133

Note: All times and temperatures are approximate. Following heat treatment at higher temperatures, amounts of sterile water in excess of manufacturer's recommendations were added to some concentrates until solubilization was visually confirmed.

* D lots manufactured by Cal Biochem; E lots manufactured by Kabi.

** Heat-treated in a dry oven (sample contained in vial).

Discussion of Selected Test Results

The results summarized in Tables I-IV can be combined to provide a relative indication of clotting activity retention and fibrinogen recovery in lyophilized plasma fractions subjected to the heat-treatment process of the present invention. More particularly, the percentage activity or measured recovery at various dilutions of reconstituted Factor VIII, Factor IX and fibrinogen concentrates can be averaged for individual control samples and compared with similarly-averaged percentage activity or measured recovery in corresponding paired samples of heat-treated Factor VIII, Factor IX and fibrinogen concentrate. Where such comparisons are made, it can be seen, for example, that lyophilized Factor VIII concentrate obtained from one manufacturer (Lot No. A C-1081) and heated at 60° C. for 10 hours retained greated than 90% of its in vitro Factor VIII clotting activity in comparison to an unheated control. The reconstituted, heat-treated Factor VIII concentrate further exhibited an

absorbance of 0.30 at 580 nm in comparison to an absorbance of 0.20 for the unheated control, and showed no differences relative to the unheated control following immunoelectrophoresis with the goat antihuman serum. Reconstituted Factor VIII concentrates from a different lot (Lot No. A NC-8747) of the same manufacturer, which had been heated in lyophilized form at 62° - 64° C. for approximately 16 hours and then stored at 6 C. for seven days, showed greater than 80% recovery of Factor VIII clotting activity in comparison to an unheated control. An overall increase in anodal migration relative to the unheated control was noted following immunoelectrophoresis against goat antihuman serum.

In similar fashion, lyophilized Factor VIII concentrate obtained from a second manufacturer (Lot No. C Al-1120), when heated at approximately 78° C. for 21 hours, showed 62% in vitro retention of clotting activity upon reconstitution as compared to an unheated control. Reconstituted Factor VIII concentrate from the same lot of the second manufacturer, after heat treatment in lyophilized form for 20 hours at approximately 80° C. still retained 57% in vitro clotting activity as compared to an unheated control, whereas reconstituted Factor VIII from the same lot of the second manufacturer, which had previously been heated in lyophilized form for one and one-half hour at approximately 100° C., retained approximately 52% in vitro clotting activity as compared to an unheated control. When a different lot (Lot No. C Al-1150) of lyophilized Factor VIII concentrate from the second manufacturer was heat-treated in accordance with the present invention, in vitro recoveries of clotting activity in comparison to the unheated control ranged from 67% for 26 hours and 20 minutes of heat treatment at 65° C. to 34% for 24 hours of heat treatment at 83° C. to 55% for 24 hours of heat treatment at 85° C. Measurements of Factor VIII antigen for the two samples of Factor VIII concentrate heat-treated at 83° C. and 85° C. respectively

showed a 15% loss and no loss in antigen levels. It should also be noted that greatly improved solubilization of the latter sample of heat-treated Factor VIII concentrate was achieved by adding between 50 ml and 75 ml of sterile water to the sample rather than the 25 ml recommended by the manufacturer.

Heat treatment of lyophilized Factor VIII samples obtained from a third manufacturer (Lot No. AHF-355) confirmed results obserbed for the first two manufacturers. That is, heat treatment of the third manufacturer's lyophilized Factor VIII concentrate for 20 hours at 64° C. yielded clotting activity recovery of 61% in comparison to an unheated control, heat treatment of the same concentrate for 17 hours at 74° C. yielded clotting activity recovery of 63% and heat treatment of the same concentrate for 17 hours at 76° C. yielded clotting activity recovery of 57%. Factor VIII antigen levels in the reconstituted samples heated at 64° C. and 74° C showed no decrease when compared to the unheated control level.

A sample of lyophilized Factor IX concentrate obtained from the first manufacturer (Lot No. A 9-C0044) and immersed in a water bath at 100° C. for 20-30 minutes yielded essentially full in vitro recovery of clotting activity when compared to an unheated control. Factor II and Factor VII both appeared stable 2 hours following reconstitution of the heat-treated sample, while Factor IX decreased approximately 20% within 6 days of reconstitution. Absorbance measurements obtained 2 hours after reconstitution yielded values of 0.006 to 0.007 at 580 mm for both control and heat-treated samples. No visual difference could be detected between the heat-treated concentrate and the unheated control following immunoelectrophoresis of the Factor IX concentrate against goat anti-human serum. Additional samples of Factor IX concentrate from the first manufacturer, which were respectively heat-treated in lyophilized form at 100° C.

for 12 hours and at 110° C. for 13 hours, also showed full recovery of Factor IX clotting activity, although complete solubilization of the latter sample required 40 ml to 60 ml greater of sterile water as opposed to the manufacturer's recommended 20 ml. The data from Table III thus suggests that Factor IX concentrate in lyophilized form is largely heat stable for 4 hours at temperatures between 100° C.-110° C.

A sample of lyophilized fibrinogen concentrate obtained from a fourth manufacturer (Lot No. D-003678) and heat-treated for 11 hours at 60° C. showed no in vitro loss of fibrinogen when compared with an unheated control. A sample of lyophilized fibrinogen concentrate obtained from the same manufacturer, when heat-treated for 17 hours at 60°C., showed a fibrinogen recovery of 97% compard with the unheated control. Samples of lyophilized fibrinogen concentrates obtained from a fifth manufacturer (Lot E), when heated for 10 and 23 hours respectively at 60° C. and 65° C., showed no in vitro loss of fibrinogen relative to the unheated control, while a sample of lyophilized fibrinogen concentrate from the fifth manufacturer showed 74% fibrinogen recovery compared to the control following heat treatment for 3 hours at 100° C.

As previously indicated, in vivo testing of heat-treated Factor VIII and Factor IX concentrates was carried out using hemophilia A or Factor VIII deficient and hemophilia B or Factor IX deficient dogs. The hemophilia A dog received reconstituted Factor VIII concentrate which had previously been heat-treated in lyophilized form at 60° C. for 10 hours, while the hemophilia B dog received reconstituted Factor Ix concentrate which had previously been heat-treated at 100° C. for 3 to 4 hours. Results of the in vivo testing are reported in Tables V and VI below.

TABLE V

F-VIII Deficient Dog Given Heat Treated Factor VIII Concentrate

	HCT %	Protein gm %	WBC /mm³	Platelets /mm³	FVIII RA %*	FVIII C %*	Temp °F	Respi-ration	Pulse
PRE	44	6.1	3,795	330,000	106	<2 (1-2)	100.0	48	132
Infusion	20 ml given in 3.5 min					400*			
15 min	45	5.9	3,190	110,000	151	12	102.3	42	138
90 min	47	6.0	6,050	165,000	n=2 166, 159	12	101.0	pant	108
3 hours	44	6.1	5,115	231,000	168	15	100.9	pant	108
5 hours	43	6.0	4,785	165,000	159	9	100.5	30	114
7.5 hours	44	5.9	3,245	198,000	150	9	101.0	42	108

TABLE VI

F-IX Deficient Dog Given Heat Treated Factor IX Concentrate

	HCT %	Protein gm%	WBC /mm³	Platelets /mm³	Thrombin clot time sec	FVIII C %*	F-IX %*	Temp °F	Respi-ration	Pulse
PRE	43	5.5	6,840	187,000	5.5	67	<1 (0-5-1)	101.5	36	160
Infusion	20 ml given in 3 min.					5*	524*			
15 min	42	5.6	6,005	429,000	5.5	47	9	101.8	30	160
90 min	44	5.8	6,545	253,000	5.5	59	10	102.0	48	144
3 hours	40	5.7	8,910	429,000	6.0	39	6		42	162
5 hours	41	5.8	5,610	231,000	6.0	64	6	100.3	42	126
7.5 hours	44	5.7	6,985	363,000	5.5	95	4	101.0	36	162

The results reported in Table V amply illustrate the marked increase in Factor VIII clotting activity for a hemophilia A dog following injection of heat-treated Factor VIII concentrate. Similarly, the results reported in Table VI amply illustrate the Factor IX recovery observed in a hemophilia B dog following injection of heat-treated Factor IX concentrate. The apparent absence of physiological stress or other adverse reaction as seen from the data in Tables V and VI suggests that Factor VIII and Factor IX concentrates which have been processed according to the steps of the present invention remain biologically acceptable.

It has now been demonstrated that plasma fractions such as Factor VIII and Factor IX concentrates of varying purity can be safely heat-treated in lyophilized form at elevated temperatures for extended periods of time without significantly destroying the clotting activity of the concentrates. It has further been demostrated that plasma fractions such as fibrinogen of varying purity can be safely heat-treated in lyophilized form at elevated temperatures for extended periods of time without destroying the recoverability of the fibrinogen. Visual obvervations confirm that the solubility of lyophilized Factor VIII, Factor IX and fibrinogen concentrates is not deleteriously affected by heat-treatment inasmuch as the amount of sterile water added to the lyophilized concentrate samples during reconstitution can simply be increased until complete solubility is achieved. Consequently, through suitable adjustment of the heating temperature, length of heating and purity levels involved, hepatitis virus of both the B type and the non-A, non-B type can be inactivated in plasma fractions while maintaining the viability and therapeutic integrity of the fractions. Given the additional fact that hepatitis B virus and probably non-A, non-B hepatitis virus are preferentially distributed in the clotting factor

fractions, i.e., in Factor VIII and Factor IX concentrates, heat treatment of the clotting factor fractions in lyophilized form at suitable temperatures for suitable periods of time can also serve to render the hepatitis virus immunogenic as well as non-infectious. As a consequence, reconstituted heat-treated lyophilized Factor VIII and Factor IX concentrates can function as hepatitis vaccines while simultaenously providing the therapeutic benefits otherwise associated with clotting factor fractions.

It should, moreover, be apparent from extrapolation of the test results reported in Tables I-VI that the method of the present invention can be performed at essentially any point during the plasma fractionation process. That is, at any point along the fractionation process where a plasma derivative can be lyophilized, heat treatment of the plasma derivative can be performed and the derivative resolubilized or reconstituted prior to continuation of the fractionation process. Thus, for example, where Factor VIII concentrate is ultimately derived from a plasma fractionation scheme such as that disclosed in Mammen, et al., "Treatment of Bleeding Disorders with Blood Components," Reviews of Hematology, Vol 1, p. 144 (1980), cryoprecipitate obtained from fresh frozen plasma, clarified extract obtained from cryoprecipitate and supernatant obtained from clarified extract can all be lyophilized and heat-treated in the same manner as the Factor VIII concentrate itself. Selection of an appropriate point in the plasma fractionation scheme for applying the heat treatment can then be based on pragmatic considerations such as cost or convenience.

Within other exemplary embodiments of the invention are the inactivation of the undesirable microorganisms such as a virus related to AIDS, namely Acquired Immune Deficiency Syndrome, cytomegalovirus and Epstein-Barr virus.

Furthermore different drying techniques could be employed to the plasma compositions such as plasma fractions, namely spray drying or vacuum drying.

The addition of sterile water to dry or lyophilized plasma in degrees greater than the volumes suggested by manufacturers creates plasma solutions of greater dilution than normally recommended; however, the effectiveness of the plasma and its constituents is not noticeably deteriorated. This is particularly useful where the temperatures to which the plasma composition is to be heated to non-activate undesirable microorganisms, such as viruses, is so high that clotting of the reconstituted plasma would normally occur unless the excess sterile water is added.

A particularly significant use of the process herein is the treatment of AHF-enriched compositions. Such compositions contain concentrations of AHF on a total protein weight basis that exceed those concentrations found in plasma or other conventional plasma protein fractions such as prothrombin complex (Factor IX concentrates), gamma globulin, albumin, fibrinogen or antithrobmin III. Such high AHF concentrations are needed to ensure that when the compositions are dissolved in water or other physiologically acceptable carrier that they can correct the patient's clotting abnormality sufficiently to demonstrate a favorable clincal effect, without requiring the infusion of excess water or extraneous proteins. Generally, a therapeutically effective dose of AHF can be determined readily by those skilled in the art. It will depend upon the clinical state of the patient, particularly the type of bleed encountered. Ordinarily, sufficient AHF should be infused to produce a patient plasma AHF level of at least 30% of that present in normal individuals, although for serious hemorrhages levels of 50% or more are preferred. This may be accomplished by infusion of a total number of AHF units as defined by the formula: Units required = body weight (kg)

x 0.4 x desired AHF increase (in % of normal).

The AHF concentration in the infusate should preferably not exceed 34 units/ml, but should be greater than 15 units/ml. If the concentration exceeds 34 units/ml, no more than 2 ml per minute should be infused. Infusated with less than 34 AHF units can be infused more rapidly, on the order of 10 to 20 ml over a 3 minute period. An AHF unit is defined as the activity of AHF present in 1 ml of normal pooled human plasma less than 1 hour old.

The AHF-enriched compositions ordinarily will contain greater than about 20 AHF units usually greater than about 300 AHF units/gram of protein. Obviously, the greater the AHF purity the more desirable the product. Compositions containing about from 100 to 2000 AHF units/gram of protein are most feasible commercially.

The AHF compositions may be freed of other plasma proteins to varying degrees during the purification process, depending upon the process used. Such plasma proteins include the blood clotting enzymes (herein meaning both the inactive proenzymes and the activated enzymes) such as prothrombin complex or Factor IX concentrate (Factors II, VII, IX and X). Factors XII or XIII, fibrinogen, albumin and gamma globulin. When an AHF composition is essentially free of blood clotting enzymes it contains subtherapeutic activities of the enzymes. The AHF compositions may be purified to a level in which the activity of any blood clotting enzyme is at a trace level, generally less than about 15 units/gram of protein, and preferably less than about 5 units/gram of protein. The ratio of AHF units to any individual enzyme unit activity in such compositions ordinarily ranges from about 10:1 to 500:1, and is preferably greater than 300:1. A unit of enzyme activity for each of the various clotting enzymes in their activitated or proenzyme forms are defined in PCT International Application WO 82/03871 (published November 11, 1982).

The heat-treated AHF compositions herein are principally administered to patients having a congential deficiency of AHF. These patients fail to synthesize biologically active AHF, and are to be distinguished from those having acquired AHF inhibitors (probably antibodies) which also cause the symptoms of hemophilia. The latter group is preferrably treated with activated clotting enzyme compositions. The clotting enzyme compositions contain therapeutically effective amounts of the enzymes. However, the compositions herein generally will not contain such amounts of blood clotting enzymes, but instead essentially rely upon AHF activity for hemostasis.

The improved AHF compositions herein contain such lower quantities of denatured AHF than produced using prior processes. Generally, the amount of denatured AHF is less than about 50% of the total active and denatured AHF in the composition. Preferably it ranges about from 30% to 10%. The amount of denatured AHF is equivalent to the precent loss in AHF units after the virus-infected AHF composition is subjected to the heat treatment method described herein.

The effect of heating virus-contaminated AHF in the dry state may be followed by assaying the AHF clotting activity, as described above, and the viral titer. Where it is difficult to measure biologically active viral titer, as in the case of hepatitis viruses, candidate virus such as bacteriophage, sindbis, adenovirus or EHC virus may be employed as disclosed in PCT International Application WO 82/03871 (published November 11, 1982. A predetermined titer of the candidate virus is seeded into a solution of the composition to be heat treated, the solution lyophilized and various heat treatments undertaken. One selects the point at which actual measurements or regression analysis (statistical extrapolation) indicate the desired degree of viral inactivation as the conditions which will govern heat treatment of the product. These conditions generally will be the time and temperature of

viral inactivation, although the moisture content of the composition will also be a variable that should be controlled. Time and temperature are described above. The moisture content should be below 5% by weight, ordinarily less than 1% by weight.

While some inactivation and denaturation of AHF takes place in the dry state heating process, it occurs at a lesser rate than the inactivation of even the more hardy candidate viruses or hepatitis virus. Thus, the infected AHF composition may be rendered substantially free of the virus in infectious form. This means that the titer of virus is reduced so low that infusion of therapeutic quantities of the product into a plurality of normal animal hosts for the virus will fail to produce clinical or serological evidence of infection in a host population, or will delay significantly the onset of infection in such population. Where an equivalent tissue culture assay is available to determine biological infectivity, it may be used in place of normal animal hosts as indicia of infectivity.

The mild dry state heating process herein preserves substantially all of the antigenicity of the infectious microbes, i.e. the epitopic sites on the organisms are not irreversibly denatured. This is in contrast to other methods involving covalent reactions with chemical substances, high energy irradiation or excessive heating.

The heating step may be accomplished in closed or open containers, as noted above, and is most efficaciously accomplished by simple, inexpensive techniques such as contact heating of the product containers, as in ovens or water or sand baths, or by infrared irradiation. For safety and for reasons of expense microwave heating is not preferred.

Several embodiments of the present invention have been illustrated hereinabove. It is to be understood, however, that various modifications to the temperature ranges, heating periods and purity levels set forth in

conjunction with the aforementioned embodiments can be made by those skilled in the art without departing from the scope and spirit of the present invention. It is therefore the intention of the inventor to be bounded only by the limits of the following claims.

Claims:

1. A method for treating a substantially dry composition including Factor VIII to reduce or eliminate the infectivity of the microorganisms present in the composition comprising heating the composition for a predetermined period of time at a predetermined temperature sufficient to reduce or eliminate the infectivity of the microorganisms.

2. A method for treating a substantially dry composition including Factor VIII to reduce or eliminate the infectivity of the microorganisms present in the composition comprising heating the composition for a predetermined period of time at a predetermined temperature sufficient to reduce or eliminate the infectivity of the microorganims while retaining substantially all of the antigenicity of the infectious microorganisms.

3. A method for treating a substantially dry composition including Factor VIII to reduce or eliminate the infectivity of the microorganisms present in the composition comprising heating the composition for a predetermined period of time at a predetermined temperature sufficient to inactivate the microorganisms present in the composition, and adding sterile water to the composition following completion of said heating step until solubilization of the composition is substantially achieved.

4. A method as claimed in any one of claims 1, 2, and 3 wherein the composition is a fraction.

5. A method as claimed in any one of claims 1, 2 or 3 wherein the composition is heated by a method other than microwave irradiation.

6. A method as claimed in any one of claims 1, 2 or 3 wherein the heating is by contact with a heated substance or by infrared irradiation.

7. A method as claimed in any one of claims 1, 2, or 3 wherein the composition is a cryoprecipitate.

8. A method as claimed in anyone of claims 1, 2 or 3 wherein the composition is a dry cryoprecipitate supernatant.

9. A method as claimed in either claims 1 or 2, including reconstituting the composition until solubilization of the composition is substantially achieved following said heating of the composition for said predetermined period of time at said predetermined temperature.

10. A method as claimed in claim 1, 2 or 3, wherein said composition is Factor VIII concentrate and said predetermined temperature is at least 60°C.

11. A method as claimed in claim 10, wherein said predetermined temperature falls within a range between 60°C and 100°C.

12. A method as claimed in any one of claims 1 to 3, wherein said composition includes Factor IX concentrate and said predetermined temperature is at least 100°C.

13. A method as claimed in claim 12 wherein said predetermined temperature falls within a range between 100°C. and 110°C.

14. A method as claimed in any one of claims 1 to 13 wherein said composition includes fibrinogen.

15. A method as claimed in claim 14, wherein said predetermined temperature falls in a range between 60°C. and 125°C.

16. A method as claimed in any one of claims 1 to 15 including lyophilizing the composition thereby to obtain the substantially dry composition.

17. A method as claimed in any one of claims 1, 2 or 3 wherein the infectivity of the microorganism is reduced or eliminated solely by heating the composition.

18. A method as claimed in any one of claims 1, 2 or 3 wherein the infectivity of the microorganism is reduced or eliminated by heating the composition.

19. A method as claimed in any one of claims 1, 2 or 3 wherein the composition is essentially free of blood clotting enzymes.

20. A method as claimed in claim 19 wherein the composition is essentially free of Factors II, VII, IX, X, XII and XIII or their activated forms.

21. A method as claimed in 20 wherein the composition contains less than about 15 units of any of the Factors II, VII, IX, X, XIII or XIII or their activated forms per gram of protein.

22. A method as claimed in claim 18 wherein the infectivity of the microorganism is not reduced or eliminated by the action of any toxic gas.

23. A method as claimed in claim 18 wherein the composition is free of a therapeutically sufficient concentration of a blood clotting enzyme.

24. A method as claimed in claim 19 wherein the composition contains a ratio of antihemophilic units of any blood clotting enzyme of about from 10:1 to 500:1.

25. A method as claimed in anyone of claims 1 to 24 wherein the microorganism is a virus.

26. A method as claimed in claim 25 wherein the virus is any hepatitis virus.

27. A method as claimed in claim 26 wherein the virus is human hepatitis virus.

28. A method as claimed in claim 26 wherein the virus is hepatitis B, or non-A, non-B hepatitis.

29. A method for treating a plasma fraction containing Factor VIII to substantially inactivate any hepatitis virus present in the fraction comprising lyophilizing the plasma fraction to increase the heat stability thereof and heating the lyophilized plasma fraction for a predetermined period of time at a predetermined temperature sufficient to render hepatitis virus present in the plasma fraction inactive.

30. A method for treating a plasma fraction to substantially inactivate any hepatitis virus present in the plasma fraction comprising lyophilizing the plasma fraction to increase the heat stability thereof, heating the lyophilized plasma fraction for a predetermined period of time at a predetermined temperature sufficient to inactivate hepatitis virus present in the plasma fraction, and adding sterile water to the lyophilized plasma fraction following completion of said heating step until solubilization of the lyophilized plasma fraction is substantially achieved.

31. A composition containing inactivated microorganisms and at least Factor VIII, said microorganisms having been inactivated by a method consisting essentially of heating the composition in a substantially dry state to inactivate said microorganisms, said composition being substantially free of the activate, infectious form of the microorganisms, and said Factor VIII being substantially active.

32. A composition as claimed in claim 31 wherein the inactivated microorganism is a virus.

33. A composition as claimed in claim 32 wherein the virus is any hepatitis virus.

34. A composition as claimed in 33 wherein the hepatitis virus is hepatitis B, or non-A, non-B hepatitis.

35. A composition as claimed in anyone of claims 31 to 34 wherein the composition is substantially dry.

36. A composition as claimed in claim 35 wherein the composition is lyophilized.

37. A composition as claimed in claim 35 wherein the composition is a cryoprecipitate.

38. A composition as claimed in claim 35 wherein the composition is a dry cryoprecipitate supernatant.

39. A composition as claimed in any one of claims 31 to 34 wherein the composition is solubilized in sterile water.

40. A composition as claimed in any one of claims 31 to 39 including a Factor VIII concentrate.

41. A composition as claimed in any one of claims 31 to 40 including a Factor IX concentrate.

42. A composition as claimed in anyone of claims 31 to 41 including fibrinogen.

43. A lyophilized composition containing non infectious hepatitis B virus and at least Factor VIII which composition is substantially free of said virus in the infectious form, said Factor VIII being substantially active.

44. A lyophilized composition containing non-infectious non-A, non-B hepatitis virus and at least Factor VIII, said virus having been made non-infectious by a method consisting essentially of heating the virus until the virus is inactivated, which composition is substantially free of said virus in the infectious form, said Factor VIII being substantially active.

45. A method for inactivating virus in a composition which contains at least an antihemophilic factor and which is suspected to contain an infective virus, which method for inactivating consists essentially of heating the composition in the dry state until the virus is inactivated.

46. A method for treating a dry composition containing antihemophilic factor purified to at least a greater activity of antihemophilic factor per unit weight of protein than the concentration of such factor in normal human plasma, and suspected to contain an infective virus, which method comprises heating the composition in the dry state until the virus is inactivated.

47. The method of claim 46 wherein the virus is any hepatitis virus.

48. The method of claim 46 wherein the titer of infective virus is not predetermined.

49. The method of claim 46 wherein the composition is free of cellular microorganisms.

50. A composition containing non-infectious virus, and antihemophilic factor said virus having been made non-infectious by a method consisting essentially of heating the virus until the virus is inactivated, which composition is substantially free of said virus in the infectious form and anti-hemophilic factor in the denatured form.

51. The composition of claim 50 wherein less than about 50% of the antihemophilic factor is in the denatured form.

52. The composition of claim 51 wherein about from 30% to 10% of the antihemophilic factor is in the denatured form.

53. A composition containing non-infectious virus and at least including Factor VIII in a purification to at least a greater activity of antihemophilic factor per unit weight of protein than the concentration of such factor in normal human plasma, which composition is substantially free of said virus in the infectious form and said factor in the denatured form.

54. In a method wherein a therapeutically effective amount of Factor VIII is infused into a patient, the improvement comprising infusing into said patient a composition containing non-infectious virus and a therapeutically effective amount of said factor, which composition is substantially free of said virus in the infectious

form and substantially free of said factor in the denatured form.

55. The method of claim 54 wherein the patient has a congenital hemophilia and not an acquired Factor VIII inhibitor.

56. The method of claim 54 wherein the amount of Factor VIII is greater than about 15 units/ml.

57. A composition comprising non-infectious virus and a therapeutically effective amount of Factor VIII, said composition being substantially free of said virus in the infectious form and including Factor VIII in the denatured form.

58. The composition of claim 57 wherein the Factor VIII is present in an amount greater than about 20 units/gram of protein in the composition.

59. The composition of claim 58 wherein the Factor VIII is present in an amount of about from 100 to 2000 units/gram of protein present in the composition.

60. A method for inactivating virus in a composition comprising at least Factor VIII in a therapeutically effective amount, which method comprises heating the composition in the dry state until the virus is inactivated.

61. A vaccine for hepatitis including the composition of an immunogenically effective amount of inactivated hepatitis virus in a blood plasma fraction together with a carrier therefor.

62. A vaccine for an undesirable virus including the composition of an immunogenically effective amount of inactivated virus in a blood plasma fraction together with a carrier therefor.